526 Rec'd PCT/PTO 10 NOV 2000

Prepared from FORM PTO-1390

Transmittal Letter to the United States Designated/Elected Office (DO/EO/US)

09/700072

Customer No.	026418	
Attorney's Docket No.:	GK-JEN-2074 / 500347 20059	
U.S. Application No.:		
International Application No.:	PCT/DE00/00802	
International Filing Date:	MARCH 09, 2000	09 MARCH 2000
Priority Date Claimed:	MARCH 11, 1999	11 MARCH 1999
Title of Invention:	LASER-COMPATIBLE NIR-MARKER DYES	
Applicant(s) for (DO/EO/US):	Peter CZERNEY and Frank LEHMANN	

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

[X] 1.This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.

[ ] 2.This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.

[ ] 3.This express request to begin national examination procedures [35 U.S.C. 371 (f)] at any time rather than delay examination until the expiration of the applicable time limit set forth in 35 U.S.C. 371(b) and PCT Articles 22 and

[ ] 4.A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

[X] 5.A copy of the Publication No WO 00/53678 14SEP00 ~~International Application as filed~~ [35 U.S.C. 371(c)(2)]

a) __ is transmitted herewith (required only if not transmitted by the International Bureau)

b) __ has been transmitted by the international Bureau

c) __ is not required, as the application was filed in the United States Receiving Office (RO/US)

[X] 6.A translation of the Publication No WO 00/53678 14SEP00 ~~International Application~~ into English [35 U.S.C. 371(c)(2)]

[ ] 7.Amendments to the claims of the International Application under PCT Article 19 [35 U.S.C. 371(c)(3)]

a) __ are transmitted herewith (required only if not transmitted by the International Bureau)

b) __ have been transmitted by the International Bureau

c) __ have not been made; however, the time limit for making such amendments has NOT expired.

d) __ have not been made and will not be made

[ ] 8.A translation of the amendments to the claims under PCT Article 19 [35 U.S.C. 371(c)(3)]

[X] 9.A oath or declaration of the inventor(s) [35 U.S.C. 371(c)(4)]

[ ] 10.A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 [35 U.S.C. 371(c)(5)]

Items 11. to 16. below concern other document(s) or information included:

[ ] 11.An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98

[X] 12.An Assignment document for recording. A separate cover sheet (PTO-1619A) in compliance with 37 CFR 3.28 and 3.31 is included.

[X] 13. x A FIRST preliminary amendment

. A SECOND or SUBSEQUENT preliminary amendment

[ ] 14.A substitute specification

[ ] 15.A change of power of attorney and/or address letter

[X] 16. (other items or information) Forms. PCT/IB/308 dated 14SEP00 and EP/SR PCT/ISA/210 dated 12JUL00 (attached to the publication) .

EXPRESS MAIL No.: EL 645 881 068 US Deposited: November 10, 2000

I hereby certify that this correspondence is being deposited with the United States Postal Service Express mail under 37 CFR 1.10 on the date indicated above and is addressed to: BOX PCT, Assistant Commissioner for Patents, Washington, DC 20231

Ruth Montalvo /Ruth Montalvo Date: 10 Nov 00

09/700072

532 Rec'd PCT/PTO 10 NOV 2000

U.S. Application No. (if known, see 37 C.F.R. 1.50):
International Application No.: PCT/DE00/00802

Attorney's Docket No: GK-JEN-2074 / 500347.20059

	CALCULATIONS	PTO
[X] 17. The following fees are submitted: BASIC NATIONAL FEE [37 CFR 1.492(a)(1)-(5)] [X] Search Report has been prepared by the EPO or JPO .... $860.00 [ ] International preliminary examination fee paid to USPTO [37 CFR 1.482] .... $690.00 [ ] No International preliminary examination fee paid to USPTO [37 CFR 1.482] but International search fee paid to USPTO [37 CFR 1.445(a)(2) .... $710.00 [ ] Neither International preliminary examination fee [37 CFR 1.482] nor International search fee [37 CFR 1.445(a)(2)] paid to USPTO .... $1,000.00 [ ] International preliminary examination fee paid to USPTO [37 CFR 1.482] and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT:	$860.00	

Claims	Number Filed		Number Extra	Rate
Total Claims (Prel.Amt)	13	-20		x $ 18. =
Indep. Claims	2	-03		x $ 80. =
[__] Multiple Dependent Claim(s) (if applicable)				+ $ 270. =

	CALCULATIONS	PTO
TOTAL OF ABOVE CALCULATIONS:	$860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date [37 CFR 1.492(e)]		
TOTAL OF ABOVE CALCULATIONS:	$860.00	
Reduction by ½ for filing by small entity, if applicable. Verified Small Entity Statement must be filed. [Note 37 CFR 1.9, 1.27, 1.28]	$.00	
SUBTOTAL:	$860 00	
Processing fee of $130.00 for furnishing the English Translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date [37 CFR 1.492(f)]	$.00	
TOTAL NATIONAL FEE:	$860.00	
Fee for recording the enclosed assignment [37 CFR 1.21(h)] The assignment must be accompanied by an appropriate cover sheet (PTO-1619A [37 CFR 3 28, 3 31]. $ 40.00 +	$ 40.00	
TOTAL FEE(S):	$900.00	
AMOUNTS TO BE REFUNDED OR CHARGED	REFUNDED CHARGED	$ $

(Please note the filing fee is based on the claims in the Preliminary Amendment)

[X] Check in the amount of $ 900.00 to cover the above fees is enclosed. (The Commissioner is hereby authorized to charge any additional fees required with this submission or to credit any overpayment to Deposit Account No: 50-1529.)

NOTE: Where an appropriate time limit under 36 CFR 1.494 or 1.495 has not been met, a petition to revive [37 CFR 1.137(a) or (b)] must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
Gerald H. Kiel , Esq.
Reed Smith LLP
375 Park Avenue
New York, NY 10152

Gerald H. Kiel — Gerald H. Kiel — 25,116 — November 10, 2000

Name (Tel. (212) 986-4090) — Signature — Reg. No — Date

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EXPRESS MAIL mailing label No. EL 758 808 824 US Date of Deposit February 2, 2001
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Ruth Montalvo

2 Feb 01

Ruth Montalvo Date

Docket No.:GK-JEN-2074/500347.20059

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Peter Czerney et al
Serial No.:	09/700,072
Filed:	November 10, 2000
For:	LASER-COMPATIBLE NIR-MARKER DYES

SECOND PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Supplemental to the Preliminary Amendment filed simultaneously with the application on November 10, 2000, please amend the above-identified application as follows:

IN THE SPECIFICATION:

Page 5, line 6, change "PO_{32}" to --PO_3^{2-} --.

IN THE CLAIMS

Please substitute claims 11, 12 and 13 previously submitted in the Preliminary

Amendment dated November 10, 2000 as follows:

--11. Laser-compatible NIR marker dyes according to claim 1, wherein they are used for optical labeling of proteins, nucleic acids, oligomers, DNA, RNA, biological cells, lipids, polymers, drugs or polymer particles.

12. A method for qualitative or quantitative determination of proteins, nucleic acids, oligomers, DNA, RNA, biological cells, lips, polymers, drugs or polymer particles, comprising the step of providing functional groups of the laser-compatible NIR marker dyes which are covalently linked to an OH-, NH_2- or SH-function of the substances to be determined.

13. The method according to claim 12, wherein the coupling reaction is carried out in aqueous solution.--

REMARKS

As a result of a Notice of Defective Translation dated January 4, 2001, filed concurrently herewith is a new certified English translation which now contains claims 1-10 as published (Publication No. W0 00/53678).

Based on the new translation, claims 11-13 have now been added by this amendment. Additionally, the specification has been amended to correct a minor error in the specification.

An early and favorable action on the merits is respectfully requested.

Respectfully submitted,

By: Gerald H. Kiel

Gerald H. Kiel
Reg. No. 25,116

February 2, 2001
REED SMITH LLP
375 Park Avenue
New York, NY 10152-1799
GHK:jl
Enc.

09/700072
532 Rec'd PCT/PTO 10 NOV 2000

EXPRESS MAIL mailing label No. EL 645 881 068 US Date of Deposit November 10, 2000

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Ruth Montalvo

10 Nov 00
Date

Docket No.: GK-JEN-2074/500347.20059

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:: Peter CZERNEY, Frank LEHMANN

Serial No. : Unknown (Int'l Appln. PCT/DE 00/00802)
filed March 9, 2000

Filed: Concurrently herewith

For: LASER-COMPATIBLE NIR-MARKER DYES

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please amend the above-identified application, filed simultaneously herewith, as follows:

IN THE SPECIFICATION:

Page 1, line 1, after the title line insert the headings:

--BACKGROUND OF THE INVENTION--;

--a) Field of the Invention--;

line 8, after this line insert the heading:

--b) Description of the Related Art--;

Page 3, line 6, after this line insert the heading:

--OBJECT AND SUMMARY OF THE INVENTION--;

line 7, after "It is the" insert --primary--;

Page 5, line 6, change "PO_3" to --PO_{32}--;

line 22, change "NH2" to -- NH_2 --;

Page 6, line 27, after this line insert the heading:

--BRIEF DESCRIPTION OF THE DRAWINGS --;

after this line insert the paragraph:

--In the drawings:--;

Page 7, line 12, after "ion laser);" insert --and--;

line 14, after this line insert the heading:

--DETAILED DESCRIPTION OF THE INVENTION--;

Page 13, last line, after this line insert the following paragraph:

--While the foregoing description and drawings represent the present invention, it will be obvious to those skilled in the art that various changes may be made therein without departing from the true spirit and scope of the present invention.--

IN THE CLAIMS:

Preceding "1." change "Patent Claims" to --What is claimed is:--.

Amend the claims as follows:

Claim 1, line 7 from the bottom of this claim, change "PO_3" to --PO_{32}--

Claim 2, line 2, change “characterized in that” to –wherein–.

Claim 3, line 2, change “characterized in that” to –wherein–.

Claim 4, line 2, change “characterized in that” to –wherein–.

Claim 5, line 2, change “characterized in that” to –wherein–.

Claim 6, line 2, change “characterized in that” to –wherein–.

Claim 7, line 2, change “characterized in that” to –wherein–.

Claim 8, line 2, change “characterized in that” to –wherein–.

Claim 9, line 2, change “characterized in that” to –wherein–.

Claim 10, line 2, change “characterized in that” to –wherein–.

Claim 11, line 1, change “claims 1 to 10" to –claim 1–;

line 2, change “characterized in that” to –wherein–.

Claim 12 (amended). [Method] <u>A method</u> for qualitative or quantitative determination of proteins, nucleic acids, oligomers, DNA, RNA, biological cells, lipids, polymers, drugs or polymer particles, [characterized in that the] <u>comprising the step of providing</u> functional groups of the laser-compatible NIR marker dyes <u>which</u> are

covalently linked to an OH-, NH_2- or SH-function of the substances to be determined.

Claim 13, line 1, change “Method” to –The method–; change

“characterized in that” to –wherein–.

IN THE ABSTRACT OF THE DISCLOSURE

Page 19, line 1, change “Abstract” to –ABSTRACT OF THE DISCLOSURE–;

line 2, change “invention" to --disclosure--;

line 9, change "According" to --As disclosed--;

line 10, line 10, delete "to the invention,"; same line, delete "(1)".

REMARKS

Claims 1-13 have been amended to correct their form and to eliminate multiple dependencies in order to reduce the filing fee.

The specification and Abstract of the Disclosure have also been amended to conform to U.S. format.

An early and favorable action on the merits is respectfully requested.

Respectfully submitted,

By: *Gerald H. Kiel*

Gerald H. Kiel
Reg. No. 25,116

November 6, 2000
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GHK:jl

LASER-COMPATIBLE NIR-MARKER DYES

The invention is directed to so-called laser-compatible NIR marker dyes based on polymethines for use in optical, especially fluorescence-optical, methods for determination and detection. Typical method applications are based on the reaction of dye-labeled antigens, antibodies or DNA segments with the respective complementary species. Possible uses are, for example, in medicine and pharmacology, biological and material sciences, environmental control and for detection of naturally occurring organic and inorganic microprobes, and so forth.

Polymethines have long been known as NIR markers and are distinguished by intensive absorption maxima which can easily be shifted in the NIR region (Fabian, J.; Nakazumi, H.; Matsuoka, M.: *Chem.-Rev.* 1992, 92, 1197). With a suitable substituent pattern and π-electron system, they fluoresce with sufficient quantum yield also in the NIR region. Accordingly, these compounds are commonly applied in different areas of technology as sensitizers in AgX materials, as laser dyes, and quantum counters, as indicator dyes in sensor engineering and, also importantly, as biomarkers ("Near-infrared Dyes for High Technology Applications", edited by Daehne, S., Resch-Genger, U.; Wolfbeis, O.-S., Kluwer, Academic Publishers - Dordrecht/Boston/London 1998). The number of polymethines used as biomarkers is limited. In this connection, only the following have achieved widespread commercial application heretofore: trimethine Cy3 derived from astraphloxine (DE 415 534) or the vinylogous pentamethine Cy5 and the double-vinylogous heptamethine Cy7 with absorption maxima at around 550 nm, 650 nm and 750 nm (US-PS 5,627,027). Further, the polysulfonated trimethine Cy3.5 and pentamethine Cy5.5 derived from the commercial heptamethine "Indocyanine Green" and "Cardio Green" are available (US-PS 5,569,766). Heptamethines which are aliphatically bridged in the polymethine chain have been developed by Patonay (US-PS 5,800,995). The terminal heteroaromatics deriving from indene (Fischer's base) and heteroindene are characteristic of all commercial biomarkers. When methyl-substituted cycloimmonium salts of this type are used as terminal polymethine building blocks, it is necessary to arrange at least five successive sp^2-hybridized carbon atoms (pentamethine) between the heterocycles in order to generate absorption maxima at the boundary of the NIR region.

A substantial disadvantage of the NIR polymethines in technical use as biomarkers consists in that lengthening of the polymethine chain increases the possibility of nucleophilic or electrophilic attack on the chain resulting in destruction of the π-system. Apart from the unsatisfactory thermal and photochemical stability, another substantial defect of polymethines consists in that they have no other absorption bands in the visible spectral region aside from their intensive absorption maxima and cannot be directly excited in this spectrum, particularly by argon lasers with an emission wavelength of λ_{em} = 488 nm or He-Ne lasers with λ_{em} = 633 nm or corresponding laser diodes from λ_{em} = 670 nm. In particular, biomarkers which are suitable for multiple color fluorescence assays can be excited only by discrete light sources (such as those mentioned above) predetermined by the π-system of the polymethine. In order to make such applications possible in spite of this (when using multiple color fluorescence assays it is necessary to excite different biomarkers, for example, with one of these excitation light sources, with clearly distinguishable emission maxima), the excitation of Cy5 is carried out by an argon laser, for example, in that an emission is caused by the excitation of light at the boundary of the NIR region by means of energy transfer by excitation of fluorescein → rhodamine → Texas Red → Cy5 (US-PS 5,800,996). Other possibilities for excitation of Cy5, for example, by means of an argon laser, include generation of microparticles from intrinsic fluorophores (phycobiliproteins) and extrinsic Cy5 which permit the Cy5 derivatives absorbing at 650 nm to be excited by energy cascades (Szöllösi, J.; Damjanovich, S.; Matyus, L.: *Cytometry* 1998, 34, 159).

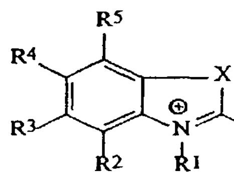
Gupta (US-PS 5,783,673) describes dye conjugates which were prepared by the reaction of phycobiliprotein with activated fluorescein, Texas Red or Cy5-dyes (phycobiliprotein/amine-reactive dye - PARD). The dye conjugates obtained in this way show additional absorption bands in the visible spectral region which can be utilized for excitation. These probes have the disadvantages of high molecular mass, uneconomical preparation and low stability of the marker dyes.

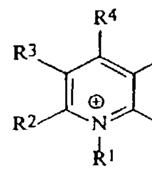
Another example for the excitation of pentamethines which are not absorbent at 488 nm is given by Glazer (US-PS 5,760,201). In addition, a strong affinity to DNA is achieved (specific ion bonding) by covalent linking with a monomethine absorbing in the desired region by way of a plurality of ammonium-

containing optimized alkyl spacers. A correspondingly complicated process for excitation is also unavoidable in this case. Further disadvantages of these marker dyes are insufficient photostability and storage stability, costly synthesis and purification steps, low absorption coefficients and unsatisfactory fluorescence quantum yields, as well as unwanted changes in optical characteristics in the presence of, or after bonding to, proteins or nucleic acid oligomers.

It is the object of the invention to provide polymethine-based NIR marker dyes with high photostability and storage stability as well as high fluorescence yields which can be excited to fluorescence in the simplest possible manner by laser radiation in the visible or near-IR spectral regions, especially by light from an argon laser, helium-neon laser or diode laser.

The invention uses polymethine-based marker dyes which contain substituted derivatives of benzooxazole, benzothiazole, 2,3,3-trimethylindolenine, 2,3,3-trimethyl-4,5-benzo-3*H*-indolenine, 2- and 4-picoline, lepidine, chinaldine and 9-methylacridine of the general formula Ia or Ib or Ic


Ia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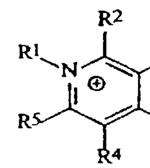
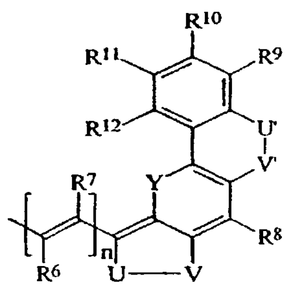

Ic

where Z is



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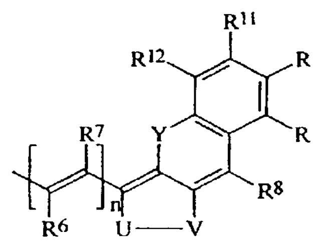

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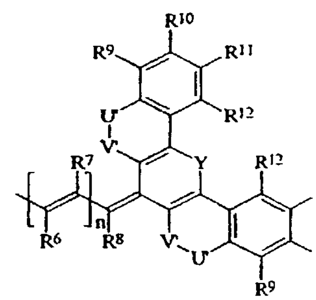

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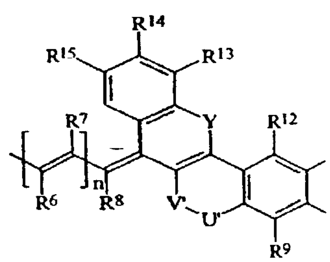
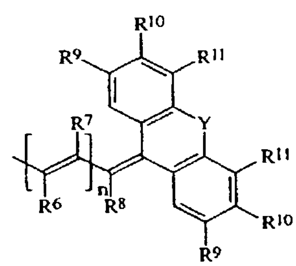




or

wherein

- X or Y is an element from the group comprising O, S, Se or the structural element N-alkyl or $C(alkyl)_2$,
- n represents the numerical value 1, 2 or 3,

- R^1 - R^{15} are identical or different and can be hydrogen, one or more alkyl- or aryl-, heteroaryl- or heterocycloaliphatic groups, a hydroxy or alkoxy group, an alkyl-substituted or cyclic amine function and/or two *ortho* groups, e.g., R^2 and R^3, together can form another aromatic ring,

- at least one of the substituents R^1 - R^{15} can be an ionizable or ionized substituent such as SO_3^-, PO_3^-, COO^- or NR_3^+ which determines the hydrophilic characteristics of these dyes,

- at least one of the substituents R^1 - R^{15} can represent a reactive group which enables a covalent linking of the dye with the carrier molecules mentioned above, and

- U-V or U'-V' are identical or different and can comprise hydrogen, a saturated aliphatic, heteroaliphatic or a lactone or thiolactone grouping.

Special embodiment forms for the marker dyes are given in subclaims 2 - 10.

These substituted indole, heteroindole, pyridine, chinoline or acridine derivatives of the general formula Ia or Ib or Ic can be used as dyes for optical labeling of organic or inorganic microparticles, e.g., proteins, nucleic acids, DNA, biological cells, lipids, drugs or organic or inorganic polymeric carrier materials. The labeling of the particles can be carried out by forming ionic interactions between the markers of the general formulas Ia or Ib or Ic and the materials to be labeled.

The functional groups of these markers which are activated relative to the nucleophiles are capable of covalent coupling to an OH-, NH2- or SH-function. This results in a system for qualitative or quantitative determination of organic and inorganic materials such as said proteins, nucleic acids, DNA, biological cells, lipids, drugs or organic or inorganic polymers.

This coupling reaction can be carried out in aqueous or predominantly aqueous solution, preferably at room temperature. A conjugate with fluorescent characteristics is formed in this way.

The compounds of the general formulas Ia or Ib or Ic and systems derived therefrom can be used in optical, especially fluorescence-optical, qualitative and quantitative determination processes for diagnosing cell characteristics, in biosensors (point-of-care measurements), genome research, and in miniaturization

technologies. Typical applications are in cytometry and cell sorting, fluorescence correlation spectroscopy (FCS), ultra-high throughput screening (UHTS), multicolor fluorescence in-situ hybridization (FISH) and microarrays (genchips).

Through the preparation of nonsymmetric polymethines which, on the one hand, as terminal function, have an easily derivable heterocycle of the pyridine, chinoline, indole, heteroindole and acridine derivative types and, on the other hand, have a novel 6-ring heterocycle, the following advantages are achieved in particular.

Even trimethines absorb in the spectral region of greater than 650 nm and show a substantially improved photochemical and thermal stability in comparison to the previously known polymethines with absorption maxima greater than 650 nm (pentamethine and heptamethine).

Molecular engineering makes it possible to control the position and intensity of the absorption maxima and emission maxima in any desired manner and to adapt the emission wavelengths of different excitation lasers, especially NIR laser diodes.

Due to the selection of suitable terminal heterocycles, the dyes according to the invention show additional absorption maxima in the visible and NIR spectral region which can be utilized for excitation, for example, with an argon laser. These dyes are particularly suited to application in multiple color fluorescence assays.

The marker dyes can be produced by means of relatively simple syntheses which are carried out in two steps and by which a large number of variously functionalized dyes, e.g., with respect to total charge of the dye and the quantity, specificity and reactivity of the activated groups used for immobilization, can be provided for specific applications.

The invention will be described more fully in the following with reference to embodiment examples shown in the drawing.

Fig. 1 shows syntheses according to embodiment examples 1 and 2;

Fig. 2 shows a synthesis according to embodiment example 3;

Fig. 3 shows syntheses according to embodiment examples 4 to 6;

Fig. 4 shows syntheses according to embodiment examples 7 and 8;

Fig. 5 shows absorption spectrum of C 1601;

Fig. 6 shows emission spectrum of C 1601 (free, bonded, 670-nm diode laser);

Fig. 7 shows syntheses according to embodiment examples 11 and 12;

Fig. 8 shows syntheses according to embodiment examples 13 and 14;

Fig. 9 shows absorption spectrum of C 1591 NHS ester;

Fig. 10 shows emission spectrum of C 1591 (free, bonded, 670-nm diode laser);

Fig. 11 shows emission spectrum of C 1591 (free, bonded, 488-nm Ar-ion laser);

Fig. 12 shows syntheses according to embodiment examples 19 and 20.

General directions for preparing 3,1-bridged 2-(2-ethoxyethenyl)-7-diethylamino-benzo[b]pyrylium perchlorates C 1595 and L 107, see Fig. 1:
0.01 mol of a 2-methylene-7-diethylamine-benzo[b]pyrylium perchlorate of formula 1a or 1b is dissolved in 40 ml acetic anhydride and briefly heated with 2.0 g triethoxymethane. The precipitate occurring after approximately one hour is sucked off and recrystallized from glacial acetic acid.

1: *6-Diethylamino-4-ethoxymethylene-1,2,3,4-tetrahydro-<dibenzo[b;e]pyrylium> perchlorate* C 1595: Yield: 3.58 g (87%); melting point:

178° C. $^{-1}$H NMR ($CDCl_3$): 1.29 (t, *J* = 7.1 Hz, 6H), 1.42 (t, *J* = 7.1 Hz, 3H), 1.78-1.82 (m, 2H), 2.54 (t, *J* = 6.0 Hz, 2H), 2.75 (t, *J* = 5.8 Hz, 2H), 3.59 (q, *J* = 7.1 Hz, 4H), 4.53 (q, *J* = 7.1 Hz, 2H), 6.93 (dd, *J* = 2.3, *J* = 9.3 Hz, 1H), 7.32 (d, *J* = 2.0 Hz, 1H), 7.50 (d, *J* = 9.3 Hz, 1H), 7.84 (s, 1H), 8.52 (s, 1H). $^{-13}$C NMR ($CDCl_3$): 12.5, 15.6, 20.2, 21.8, 27.8, 45.8, 73.1, 97.1, 108.3, 115.4, 115.8, 120.3, 130.6, 145.6, 154.8, 157.8, 163.0, 167.9. $^{-}C_{20}H_{26}ClNO_6$ (411.88): calculated C 58.32, H 6.36, Cl 8.61, N 3.40, actual C 57.75, H 6.58, Cl 8.43, N 3.46.

2: *3-Diethylamino-6-ethoxymethylene-7,8,9,10-tetrahydro-6H-<5-oxonia-cyclohepta[b]-naphthalene> perchlorate* L 107: Yield: 3.96 g (93%); melting point: 158-60° C. $^{-1}$H NMR ($CDCl_3$): 1.27 (t, *J* = 7.1 Hz, 6H), 1.39 (t, *J* = 7.1 Hz, 3H), 1.75-1.77 (m, 2H), 1.85-1.87 (m, 2H), 2.58-2.61 (m, 2H), 2.79-2.83 (m, 2H), 3.58 (q, *J* = 7.1 Hz, 4H), 4.56 (q, *J* = 7.1 Hz, 2H), 6.99 (dd, *J* = 2.4, *J* = 9.3 Hz, 1H), 7.16 (d, *J* = 2.0 Hz, 1H), 7.60 (d, *J* = 9.3 Hz, 1H), 8.00 (s, 1H), 8.18 (s, 1H). $^{-13}$C NMR ($CDCl_3$): 12.5, 15.5, 21.1, 23.8, 25.1, 29.2, 45.8, 72.4, 96.3, 113.2, 116.1, 116.3, 124.2, 130.8, 149.0, 155.0, 157.9, 162.8, 171.0. $^{-}C_{21}H_{28}ClNO_6$ (425.91): calculated C 59.22, H 6.63, Cl 8.32, N 3.29, actual C 58.76, H 6.39, Cl 8.75, N 3.34.

3: *3-Diethylamino-6-[3-(N-acetylanilino)-prop-2-ylidene]-7,8,9,10-tetrahydro-6H-<5-oxonia-cyclohepta[b]naphthalene> percholate C 1590*, see Fig. 2: 2.13 g (0.005 mol) of 2-methylene-7-diethylamine-benzo[b]pyrylium perchlorate of formula 1b are dissolved in 40 ml acetic anhydride and briefly heated with 1.29 g (0.005 mol) (3-anilinopropenylidene)-phenyl-ammonium chloride. The precipitate occurring after approximately one hour is sucked off, washed with ether and recrystallized from glacial acetic acid: Yield: 2.00 g (74%); melting point: 216-20° C. $^{-1}$H NMR (CD_3NO_2): 1.34 (t, *J* = 7.1 Hz, 6H), 1.64-1.69 (m, 2H), 1.82-1.87 (m, 2H), 2.00 (s, 3H), 2.49 (t, *J* = 6.0 Hz, 2H), 2.89 (t, *J* = 6.0 Hz, 2H), 3.72 (q, *J* = 7.1 Hz, 4H), 5.61 (dd, *J* = 11.8 Hz, *J* = 13.5 Hz, 1H), 7.04 (d, *J* = 2.4 Hz, 1H), 7.32 (dd, *J* = 2.4 Hz, *J* = 9.4 Hz, 1H), 7.36-7.39 (m, 2H), 7.54-7.65 (m, 4H), 8.21 (s, 1H), 8.27 (d, *J* = 13.5 Hz, 1H), $^{-13}$C NMR (CD_3NO_2): 12.8, 23.5, 25.2, 25.8, 25.9, 30.4, 47.2, 96.2, 109.9, 119.0, 119.3, 127.4, 129.9, 130.6, 130.7, 131.7, 132.0, 132.5, 140.1, 142.2, 151.3, 157.2, 160.1, 169.7, 171.2. $^{-}C_{29}H_{33}ClN_2O_6$ (541.04): calculated C 64.38, H 6.15, Cl 6.55, N 5.18, actual C 63.73, H 6.15, Cl 6.81, N 5.07.

General directions for preparing 3,1-bridged 2-[6-(N-acetylanilino)-hexatrien-1,3,5-ylidene]-benzo[b]pyrylium and thiopyrylium perchlorates C 1586, C 1573 and C 1574, see Fig. 3:

0.005 mol of 2-methylene-7-diethylamine-benzo[b]pyrylium perchlorate of formula 1a, 1b or a 2-methylene-4,6-diphenyl-thiopyrylium perchlorate of formula 1c are dissolved in 40 ml acetic anhydride and briefly heated with 1.42 g (0.005 mol) (5-anilinopenta-2,4-dienylidene)-phenyl-ammonium chloride. The precipitate occurring after approximately one hour is sucked off, washed with ether and recrystallized from glacial acetic acid.

4: *6-Diethylamino-4-[5-(N-acetylanilino)-penta-2,4-dienylidene]-1,2,3,4-tetrahydro-<dibenzo[b;e]pyrylium> perchlorate* C 1586: Yield: 2.65 g (96%); melting point: 246-48°C. $^{-1}$H NMR (CD_3NO_2): 1.34 (t, *J* = 7.1 Hz, 6H), 1.84-1.88 (m, 2H), 2.08 (s, 3H), 2.67 (t, *J* = 5.7 Hz, 2H), 2.85 (t, *J* = 6 Hz, 2H), 3.72 (q, *J* = 7.1 Hz, 4H), 5.38 (dd, *J* = 11.4 Hz, *J* = 13.8 Hz, 1H), 6.55 (dd, *J* = 11.9 Hz, *J* = 14.3 Hz, 1H), 7.00-7.08 (m, 2H), 7.27-7.33 (m, 3H), 7.56-7.62 (m, 3H), 7.71-7.75 (m, 2H), 8.00 (d, *J* = 13.8 Hz, 1H), 8.12 (s, 1H). $^{-}C_{30}H_{33}ClN_2O_6$ (553.05): calculated C 65.15, H 6.01, Cl 6.41, N 5.07, actual C 63.57, H 6.08, Cl 6.14, N 4.92.

5: *3-Diethylamino-6-[5-(N-acetylanilino)-penta-2,4-dienylidene]-7,8,9,10-tetrahydro-6H<5-oxonia-cyclohepta[b]naphthalene> perchlorate* C 1573: Yield: 2.61 g (92%); melting point: 202°C. $^{-1}$H NMR (CD_3NO_2): 1.36 (t, *J* = 7.1 Hz, 6H), 1.78-1.82 (m, 2H), 1.90-1.94 (m, 2H), 2.01 (s, 3H), 2.76 (t, *J* = 6 Hz, 2H), 2.95 (t, *J* = 6 Hz, 2H), 3.75 (q, *J* = 7.1 Hz, 4H), 5.39 (dd, *J* = 11.3 Hz, *J* = 13.9 Hz, 1H), 6.57 (dd, *J* = 11.9 Hz, *J* = 14.3 Hz, 1H), 6.98-7.06 (m, 2H), 7.32-7.36 (m, 3H), 7.52-7.63 (m, 4H), 7.77 (d, *J* = 9.4 Hz, 1H), 7.97 (d, *J* = 13.8 Hz, 1H), 8.22 (s, 1H). $^{-13}$C NMR (CD_3NO_2): 12.3, 22.9, 25.2, 25.5, 25.7, 30.2, 46.8, 95.7, 114.5, 118.7, 119.1, 126.0, 127.7, 129.7, 130.1, 131.1, 131.5, 132.1, 137.8, 140.1, 142.1, 144.4, 150.8, 156.9, 159.8, 169.3, 170.3, $^{-}C_{31}H_{35}ClN_2O_6$ (567.08): calculated C 65.66, H 6.22, Cl 6.25, N 4.94, actual C 64.42, H 6.27, Cl 6.13, N 4.78.

6: *8-[5-(N-Acetylanilino)-penta-2,4-dienylidene]-2,4-diphenyl-5,6,7,8-tetrahydro-<benzo[b]thiopyrylium>percholate* C 1574: Yield: 2.37 g (79%); melting point: 216-18°C. $^{-}C_{34}H_{30}ClNO_5S$ (600.13): calculated C 68.05, H 5.04, Cl 5.91, N 2.33, S 5.34, actual C 67.34, H 5.03, Cl 5.67, N 2.24, S 5.18.

General directions for preparing 3,1-bridged 7-diethylamino-2-[3-(1-alkyl-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-propen-1-yl]-benzo[b]pyrylium perchlorates C 1592 C 1601, see Fig. 4:

In a first variant, 0.005 mol of an indole derivative 2a or 2b (Mujumdar, R. T.; Ernst, L. A.; Mujumdar, S. R.; Lewis, C. J.; Waggoner, A. S.: *Bioconjugate Chem.* 1993, 4, 105) together with 2.13 g (0.005 mol) L 107 are heated under reflux for about ten minutes in 30 ml acetic anhydride and 10 drops piperidine. After cooling, the raw product is precipitated with ethyl ether and purified by column chromatography (silica gel, methanol/acetone 1:1).

In a second variant (as indicated in Fig. 4), 2.13 g (0.005 mol) of a percholate 3b (Kanitz, A.; Hartmann, H.; Czerney, P.: J. *Prakt. Chem.* 1998, 340, 34) are used instead of L 107. It is necessary to increase the reaction time by approximately ten minutes.

7: *3-Diethylamino-6-[2-(1-n-butyl-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-ethylidenl]-7,8,9,10-tetrahydro-6H-<5-oxonia-cyclohepta[b]naphthalene> percholate* C 1592: Yield/variant A: 1.87 g (63%), yield/variant B: 1.34 g (45%); melting point: 216-18°C. - HRMS-FAB ($C_{34}H_{43}N_2O$): calculated 495.337539; actual 495.335970; D = 1.569 mmU.

8: *3-Diethylamino-6-<2-[1-(4-sulfonatobutyl)-3,3-dimethyl-5-sulfanato-1,3-dihydroindol-2-ylidene]-ethylidenl>-7,8,9,10-tetrahydro-6H-<5-oxonia-cyclohepta[b]naphthalene>potassium* C 1610: Yield: 1.25 g (36%); melting point: 216-18°C. - HRMS-FAB ($C_{34}H_{42}KN_2O_7S_2$): calculated 693.207053; actual 693.203060; D = 3.99 mmU.

9: *Absorption spectra of C 1601*: Fig. 5 shows the absorption spectrum of C 1601 in pure PBS (phosphate buffer saline) and after the addition of human serum albumin (HSA).

10: *Fluorescence spectra of C 1601*: Fig. 6 shows the emission spectra of C 1601 (excited by a 670-nm diode laser) in pure PBS and after the addition of HSA. The intensity of fluorescence was increased by a factor of 5 after the addition of HSA.

General directions for preparing 3,1-bridged 7-diethylamino-2-[3-(1-(5-carboxypentyl-3,3-dimethyl--5-sulfonato-1,3-dihydro-indol-2-ylidene)-propen-1-yl]-benzo[b]pyrylium perchlorates C 1602 and C 1591, see Fig. 7:

1.77 g (0.005 mol) of an indole derivative 2c (Mujumdar, R. T.; Ernst, L. A.; Mujumdar, S. R.; Lewis, C. J.; Waggoner, A. S.: *Bioconjugate Chem.* 1993, 4, 105) and 0.005 mol of C 1595 or L 107 are heated under reflux for about ten minutes in 40 ml of a mixture of pyridine/acetic anhydride (1/1). After cooling, the raw product is precipitated with ethyl ether and purified by column chromatography (silica gel, methanol).

11: *6-Diethylamino-4-<2-[1-(5-carboxypentyl)-3,3-dimethyl-5-sulfonato-1,3-dihydro-indol-2-yliden]-ethylidenl>-1,2,3,4-tetrahydro-<dibenzo[b;e]pyrylium>betaine C 1602*: Yield: 2.20 g (71%); melting point: >310°C. -$C_{35}H_{44}KN_2O_7S$ (657.89 * H_2O): calculated C 62.20, H 6.56, N 4.14, S 4.74, actual C 61.74, H 6.53, N 4.06, S 4.26. -HRMS-FAB ($C_{35}H_{43}N_2O_6S$): calculated 619.284184; actual 619.286390; D = -2.205 mmU.

12: *3-Diethylamino-6-<2-[1-(5-carboxypentyl)-3,3-dimethyl-5-sulfonato-1,3-dihydro-indol-2-yliden]-ethylidenl>-7,8,9,10-tetrahydro-6H-<5-oxonia-cyclohepta[b]naphthalene> betaine* C 1591: Yield: 2.15 g (68%); melting point: >340°C. -$C_{36}H_{46}KN_2O_7S$ (671.91 * H_2O): calculated C 62.68, H 6.73, N 4.06, S 4.64, actual C 62.37, H 6.61, N 4.07, S 4.34. -HRMS-FAB ($C_{36}H_{45}N_2O_6S$): calculated 633.299834; actual 633.308710; D = -8.875 mmU.

General directions for preparing NHS ester with N-hydroxysuccinimide (NHS)/N,N'-dicyclohexylcarbodiimide (DCC), see Fig. 8:

15 mg of C 1602 or C 1591, 14 mg of DCC and 4 mg of NHS are dissolved in 1 ml dry DMF and mixed with 10 µl of triethylamine. The reaction mixture is stirred for 24 hours at room temperature and subsequently filtered. After extracting the solvent, the residue is washed with ether and dried in an oil pump vacuum.

13: *C 1602 NHS ester*: The reaction runs quantitatively.

14: *C 1591 NHS ester*: The reaction runs quantitatively.

15: *Covalent labeling of human serum albumin (HSA) with C 1591 NHS ester:* C 1591 NHS ester (approximately 0.5 mg) are dissolved in 50 µl of DMF and 5 mg of HSA are dissolved in 750 µl of bicarbonate buffer (0.1 mol/l, pH = 9.0). Both solutions are gradually combined and stirred for 20 hours at room temperature. The labeled HSA is then separated from the unattached dye by gel chromatography. Sephadex G50 is used as stationary phase, phosphate buffer (22 mmol/l, pH 7.2) is used as solvent.

16: *Absorption spectra of C 1591 derivatives*: Fig. 9 shows the absorption spectrum of an activated C 1591 NHS ester and C 1591 covalently bonded to HSA. PBS (phosphate buffer saline) was used as solvent for both measurements.

17: *Fluorescence spectra of C 1591 derivatives*: Fig. 10 shows the emission spectrum of an activated C 1591 NHS ester and C 1591 covalently bonded to HSA. A 670-nm diode laser (*Spindler & Hoyer*, maximum output 3 mW) was used for excitation. PBS was used as solvent for both measurements.

18: *Fluorescence spectra of C 1591 derivatives*: Fig. 11 shows the emission spectrum of an activated C 1591 NHS ester and C 1591 covalently bonded to HSA. A 488-nm Ar-ion laser (*Ion Laser Technology*, maximum output 100 mW) was used for excitation. PBS was used as solvent for both measurements.

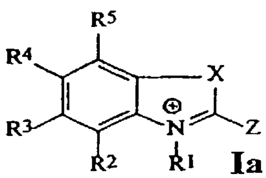
19: *3-Diethylamino-6-<2-[1-(3-acetoxypropyl)-3.3-dimethyl-1.3-dihydro-indol-2-yliden]-ethyliden>7.8.9.10-tetrahydro-6H-<5-oxonia-cyclohepta[b]naphthalen>perchlorate C 1594, see Fig. 12*: 1.94 g (0.005 mol) of 1-(1-acetoxypropyl)-2,3,3-trimethyl-3*H*-indolinium iodide 2 d (Brush et al., US-PS 5,808,044) and 2.13 g (0.005 mol) of L 107 are heated under reflux for approximately 20 minutes in a mixture of 20 ml pyridine and 20 ml acetic anhydride. After cooling, the intermediate stage which is still acetylated is precipitated with ether and dried under vacuum. The product is purified by preparative column chromatography (silica gel, methanol). Yield: 0.87 g (29%); melting point 155-62°C. - HRMS-FAB ($C_{35}H_{43}N_2O_3$): calculated 539.327368; actual 539.328510; D = -1.142 mmU.

20: *Preparation of C 1594 phosphoramidite, see Fig. 12*: For hydrolysis, 200 mg of C 1594 are dissolved in 10 ml methanol and stirred for two

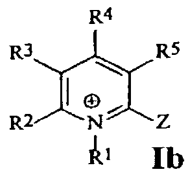
hours while adding 50 mg of sodium carbonate, followed by filtration and the deacylated dye is precipitated by addition of ether and dried. The obtained product is dissolved in dry DMF and mixed with 0.15 ml of N,N-diisopropylamine. 40 μl of 2-cyanoethyl-N,N,-diisopropylchlorophosphoramidite are added to this solution three times over the course of an hour. The reaction is tracked by thin-film chromatography and after quantitative running of the reaction the product is used directly for labeling DNA.

Patent Claims

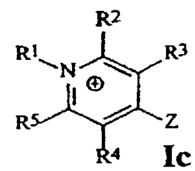
1. Laser-compatible NIR marker dyes based on polymethine, containing substituted derivatives of benzooxazole, benzothiazole, 2,3,3-trimethylindolenine, 2,3,3-trimethyl-4,5-benzo-3*H*-indolenine, 2- and 4-picoline, lepidine, chinaldine and 9-methylacridine of the general formula Ia or Ib or Ic



Ia

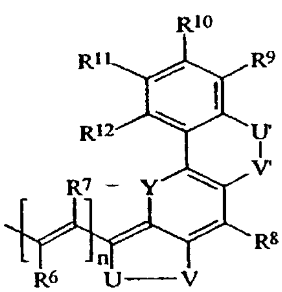


Ib



Ic

where Z is



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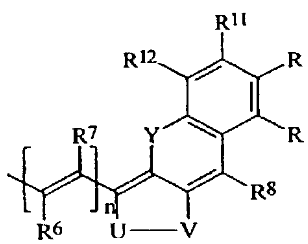

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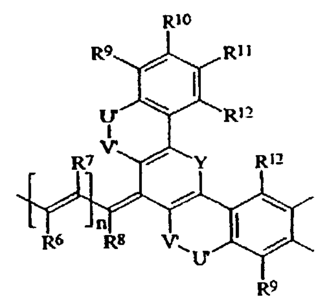

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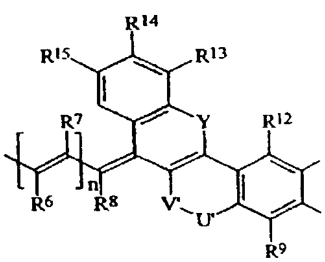
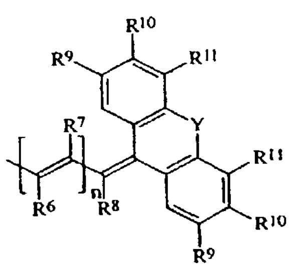




or

wherein

- X or Y is an element from the group comprising O, S, Se or the structural element N-alkyl or C(alkyl)$_2$,
- n represents the numerical value 1, 2 or 3,
- R^1 - R^{15} are identical or different and can be hydrogen, one or more alkyl- or aryl-, heteroaryl- or heterocycloaliphatic groups, a hydroxy or alkoxy group, an alkyl-substituted or cyclic amine function and/or two *ortho* groups, e.g., R^2 and R^3, together can form another aromatic ring,
- at least one of the substituents R^1 - R^{15} can be an ionizable or ionized substituent such as SO_3^-, PO_3^-, COO^- or NR_3^+ which determines the hydrophilic characteristics of these dyes,
- at least one of the substituents R^1 - R^{15} can represent a reactive group which enables a covalent linking of the dye with the carrier molecules mentioned above, and
- U-V or U'-V' are identical or different and can comprise hydrogen, a saturated aliphatic, heteroaliphatic or a lactone or thiolactone grouping.

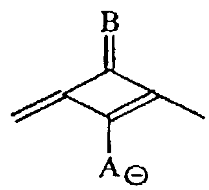
2. Laser-compatible NIR marker dyes according to claim 1, characterized in that the reactive group is selected from the following functionalities: isothiocyanates, monochlorotriazines, dichlorotriazines, aziridines, sulfonyl halides, *N*-hydroxysuccinimide ester, imido esters, glyoxal or aldehyde for amine and hydroxy functions or maleimides or iodacetamide for thiol functions and phosphoramidites for labeling DNA or RNA or fractions thereof.

3. Laser-compatible NIR marker dyes according to claim 1, characterized in that the reactive group is bonded to the actual chromophore via spacer groups of the general structure $-(CH_2)_m-$, wherein m can have values from 1 to 18.

4. Laser-compatible NIR marker dyes according to claim 1, characterized in that the structural unit $=CR^7$- also contains a bridge over four-, five-

and six-member ring systems, wherein reactive groups are also located at the latter and substituents A-G can have the same functionality as substituents R^1-R^{15}.

5. Laser-compatible NIR marker dyes according to claim 4, characterized in that the structural unit $=CR^7$- (n = 2) represents



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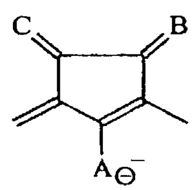
6. Laser-compatible NIR marker dyes according to claim 4, characterized in that the structural unit $=CR^7$- (n = 2) represents



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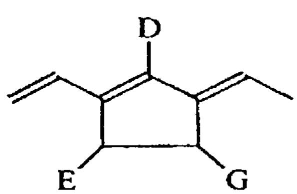
7. Laser-compatible NIR marker dyes according to claim 4, characterized in that the structural unit $=CR^7$- (n = 3) represents



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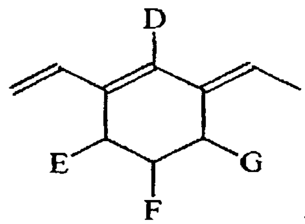
8. Laser-compatible NIR marker dyes according to claim 4, characterized in that the structural unit $=CR^7-$ (n = 3) represents



9. Laser-compatible NIR marker dyes according to claim 4, characterized in that substituents A-C represent O, S, C(CN)2 or N-R, wherein R in N-R can represent an aliphatic or aromatic or reactive aliphatic or aromatic group such as $(CH_2)_nCOOH$ or $(CH_2)_nNH_2$.

10. Laser-compatible NIR marker dyes according to claim 4, characterized in that substituent D represents Cl or an aromatic or aliphatic ring system on which reactive substituents corresponding to R^1 to R^{15} are possibly arranged.

Abstract

The invention is directed to so-called laser-compatible NIR marker dyes based on polymethines for use in optical, especially fluorescence-optical, methods for determination and detection, for example, in medicine, pharmacology, biological, material and environmental sciences. It was the object of the invention to provide polymethine-based NIR marker dyes with high photostability and storage stability as well as high fluorescence yields which can be excited to fluorescence in the simplest possible manner by laser radiation in the visible or NIR spectral region, especially by light from an argon laser, helium-neon laser or diode laser. According to the invention, dyes based on polymethines of the general formula (I) are used.

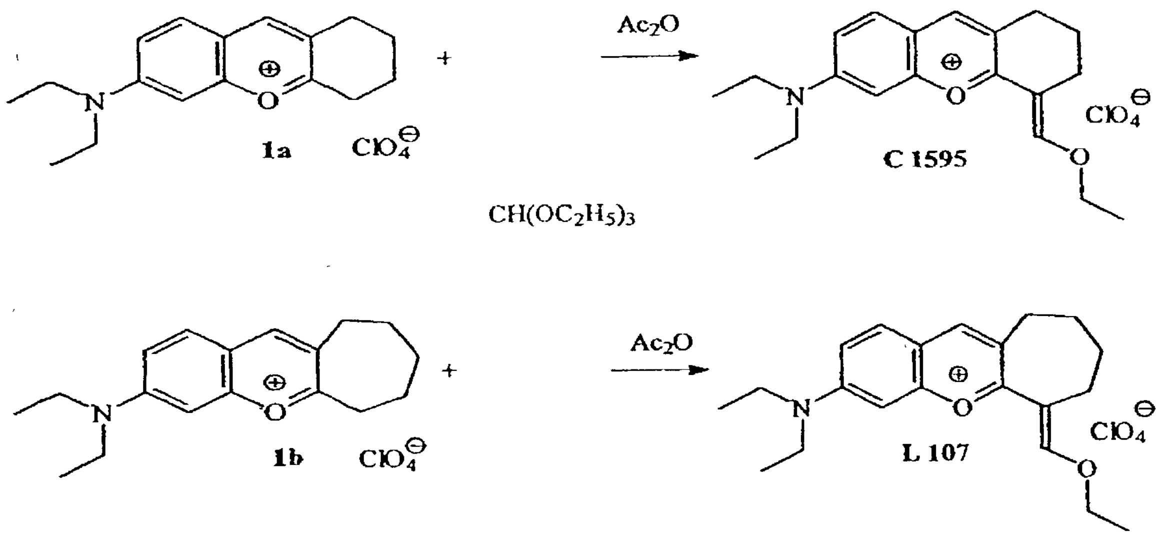

Ac$_2$O
1a
$ClO_4^{\ominus}$
C 1595
$ClO_4^{\ominus}$
$CH(OC_2H_5)_3$
Ac$_2$O
1b
$ClO_4^{\ominus}$
L 107
$ClO_4^{\ominus}$

Fig. 1

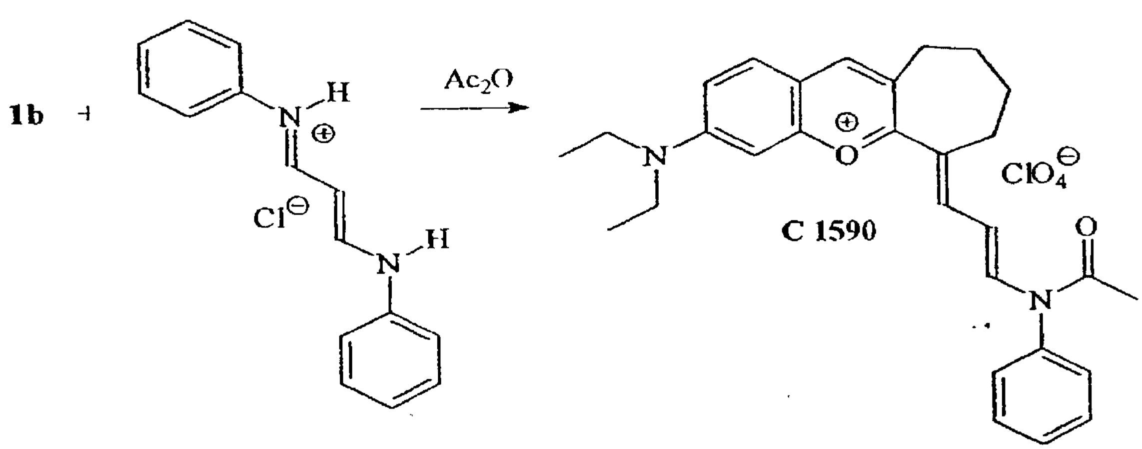

1b
Ac$_2$O
$Cl^{\ominus}$
C 1590
$ClO_4^{\ominus}$

Fig. 2

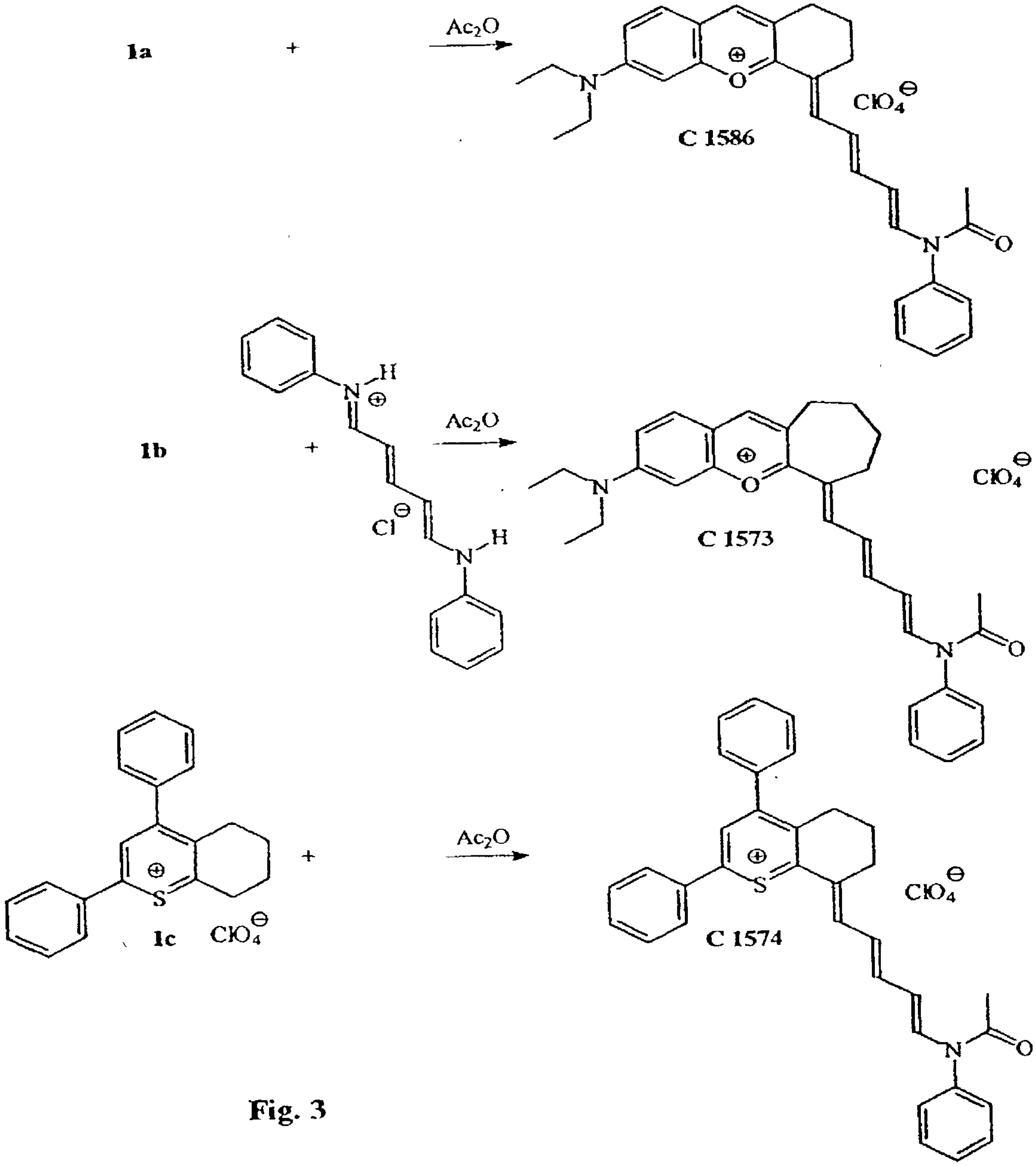

1a
+
Ac_2O
$ClO_4^{\ominus}$
C 1586
1b
+
$Cl^{\ominus}$
Ac_2O
$ClO_4^{\ominus}$
C 1573
1c
$ClO_4^{\ominus}$
+
Ac_2O
$ClO_4^{\ominus}$
C 1574

Fig. 3

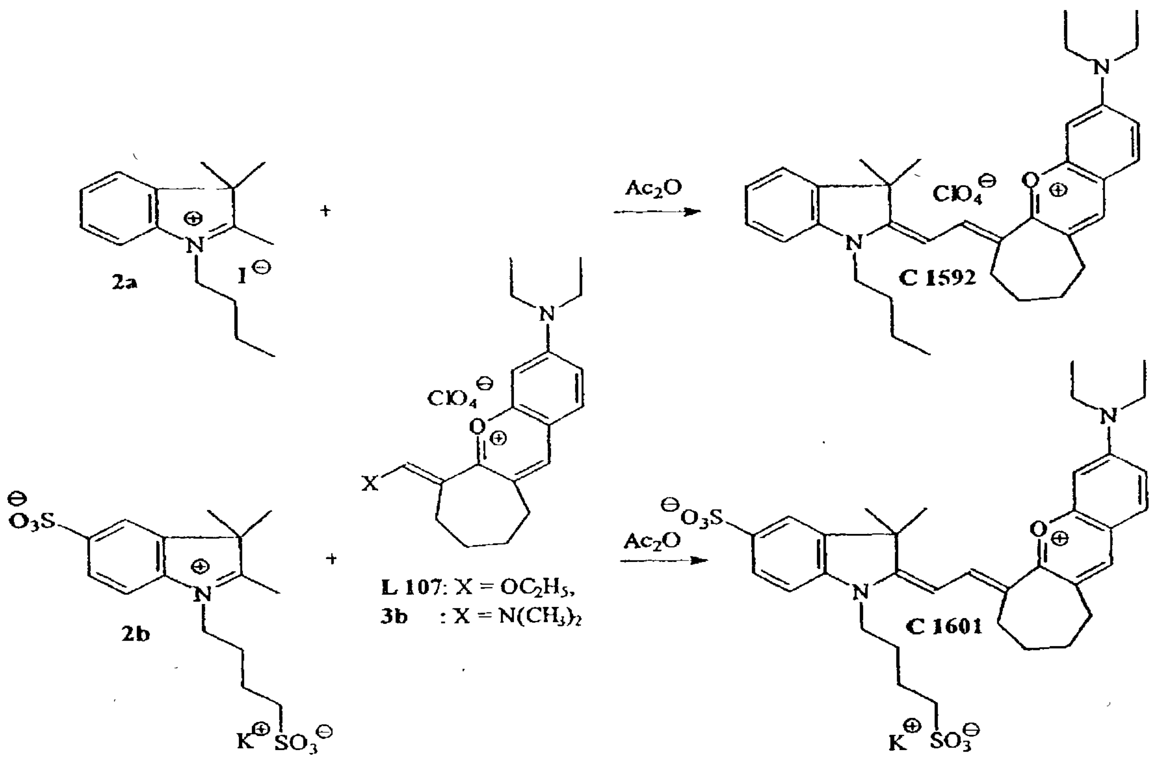

2a
$I^{\ominus}$
Ac_2O
$ClO_4^{\ominus}$
C 1592
$ClO_4^{\ominus}$
X
L 107: X = OC_2H_5,
3b : X = $N(CH_3)_2$
$^{\ominus}O_3S$
2b
$K^{\oplus}$ $SO_3^{\ominus}$
Ac_2O
C 1601

Fig. 4

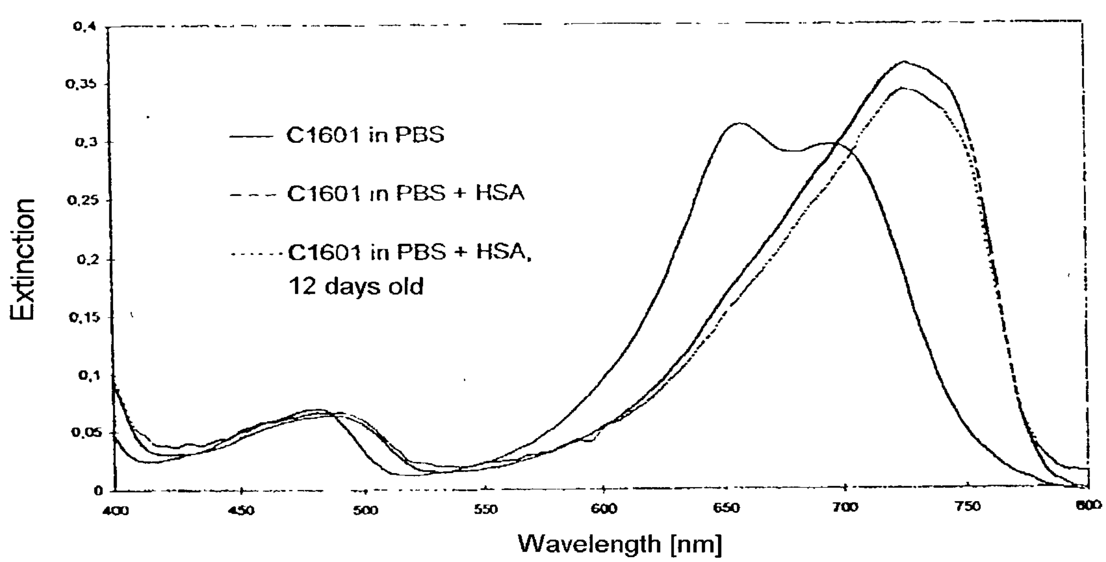

C1601 in PBS
C1601 in PBS + HSA
C1601 in PBS + HSA,
12 days old
Extinction
Wavelength [nm]

Fig. 5

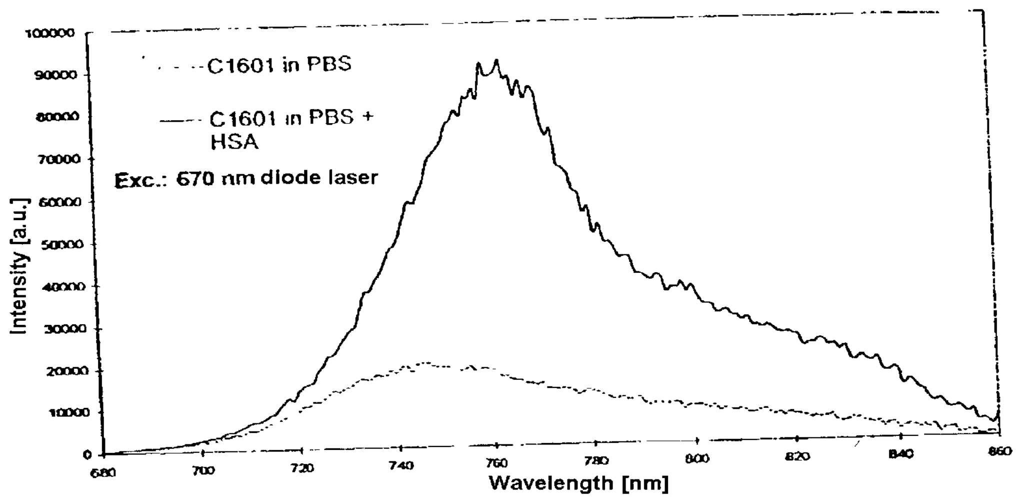

C1601 in PBS
C1601 in PBS + HSA
Exc.: 670 nm diode laser
Intensity [a.u.]
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
680
700
720
740
760
780
800
820
840
860
Wavelength [nm]

Fig. 6

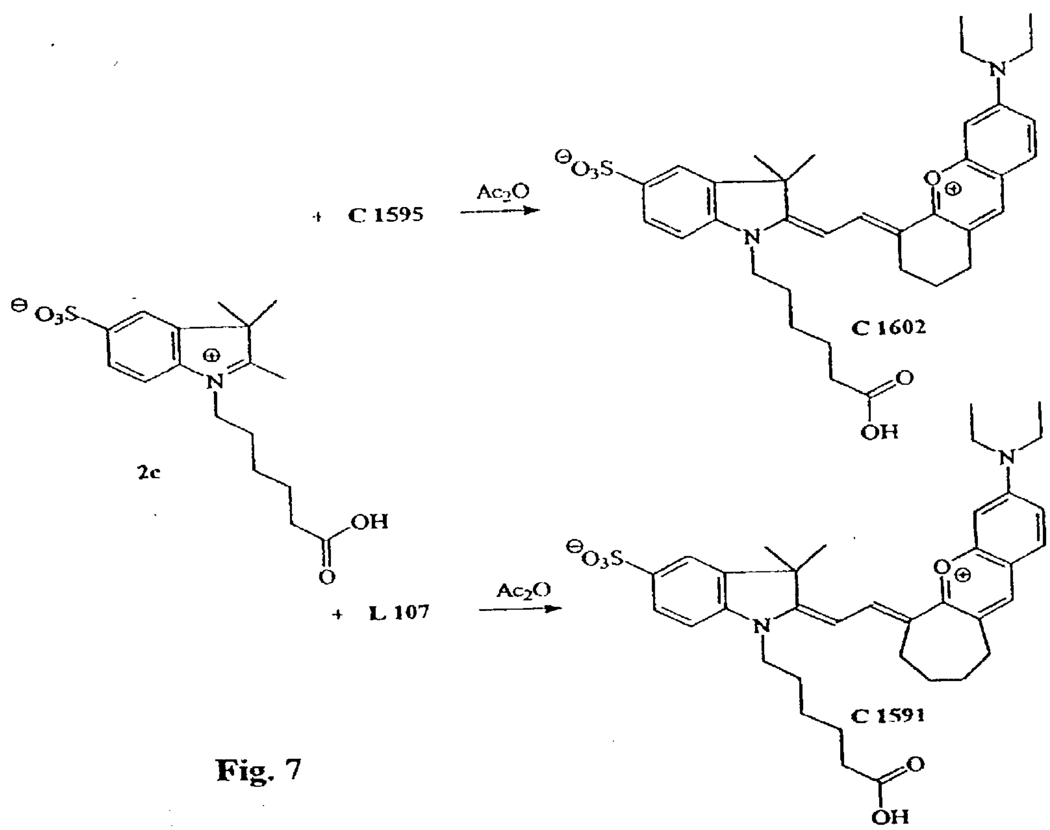

+ C 1595
Ac_2O
C 1602
2c
OH
O
+ L 107
Ac_2O
C 1591

Fig. 7

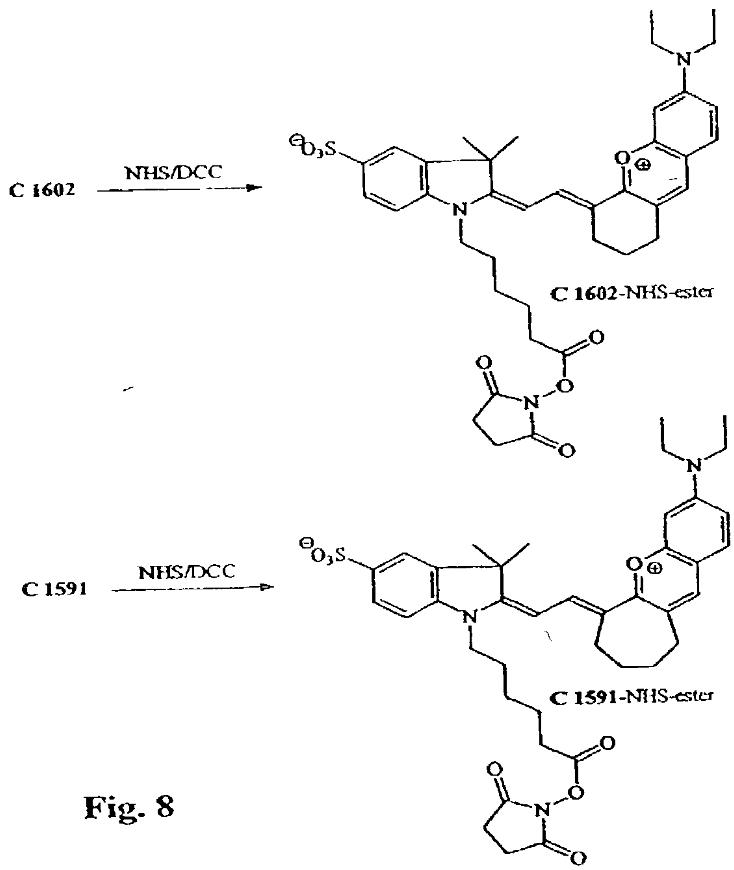

C 1602
NHS/DCC
C 1602-NHS-ester
C 1591
NHS/DCC
C 1591-NHS-ester

Fig. 8

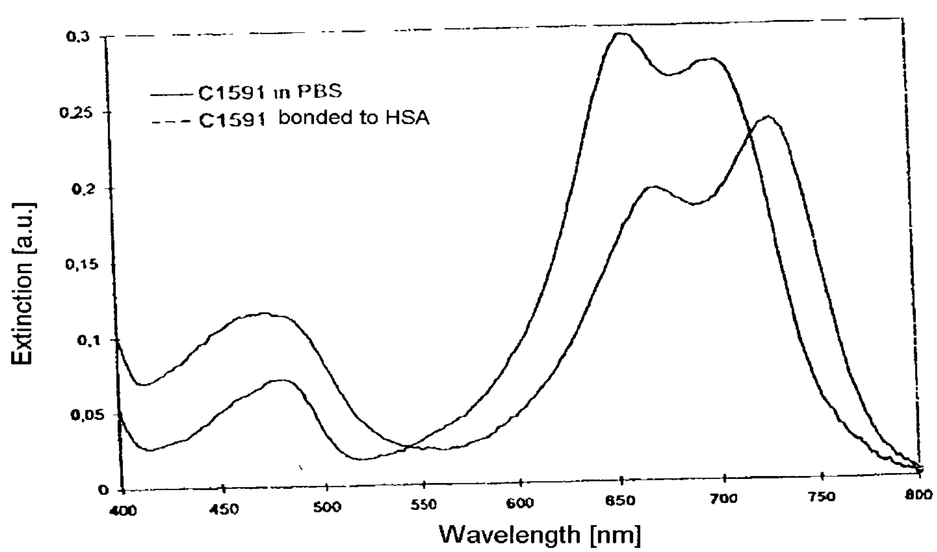

C1591 in PBS
C1591 bonded to HSA
Extinction [a.u.]
Wavelength [nm]

Fig. 9

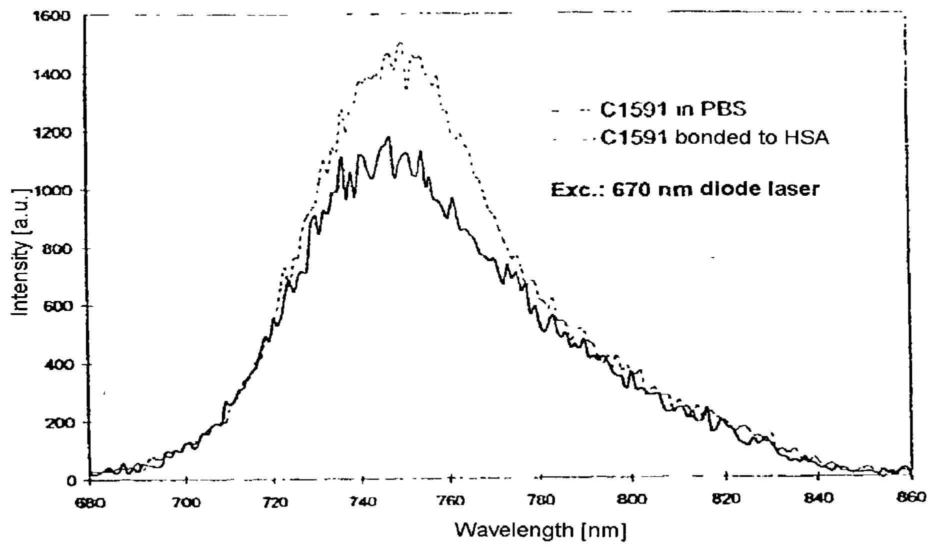

C1591 in PBS
C1591 bonded to HSA
Exc.: 670 nm diode laser
Intensity [a.u.]
Wavelength [nm]
0
200
400
600
800
1000
1200
1400
1600
680
700
720
740
760
780
800
820
840
860

Fig. 10

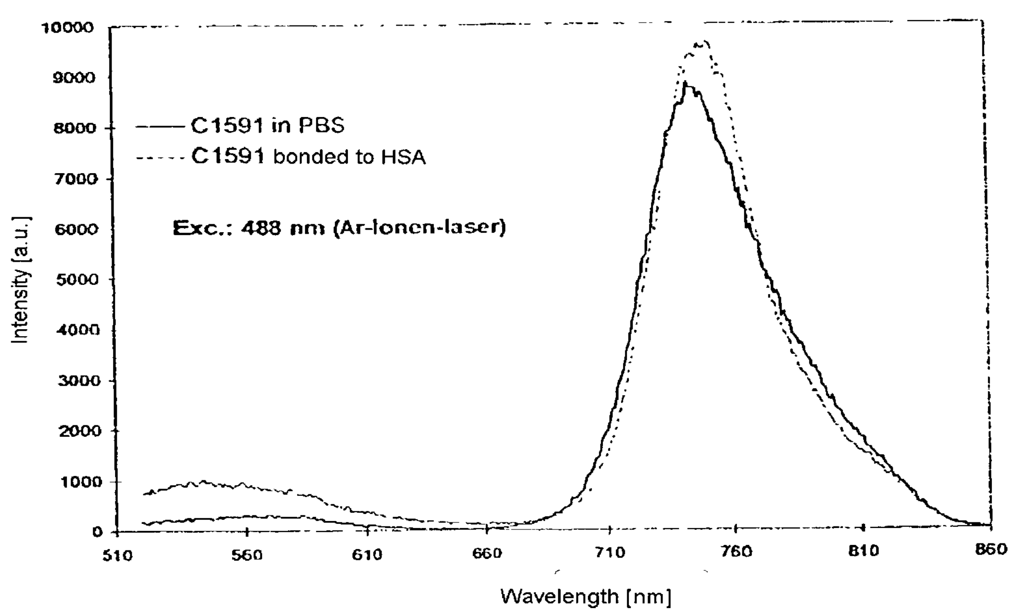

C1591 in PBS
C1591 bonded to HSA
Exc.: 488 nm (Ar-Ionen-laser)
Intensity [a.u.]
Wavelength [nm]
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
510
560
610
660
710
760
810
860

Fig. 11

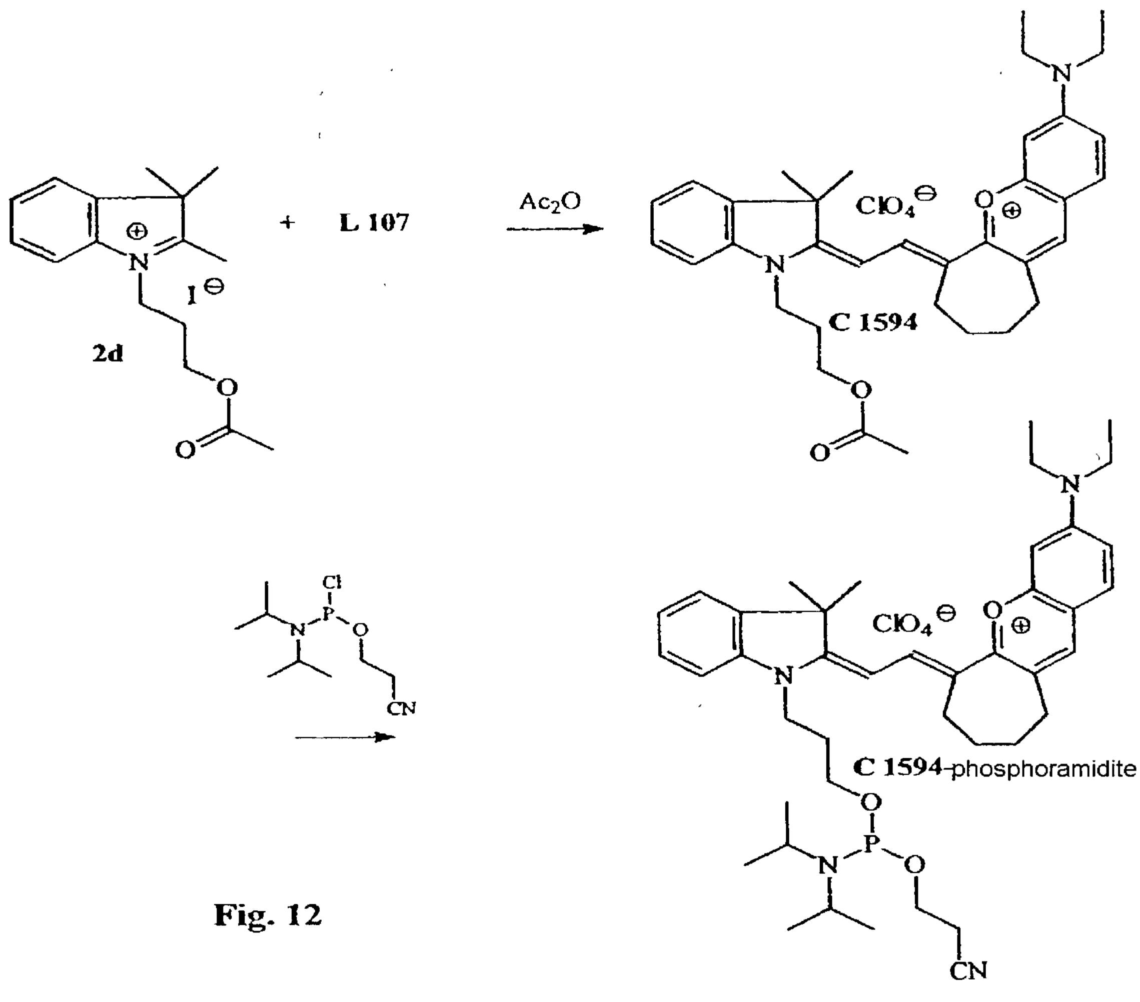


Fig. 12

UNITED STATES OF AMERICA COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION	FILE NO. GK-JEN-2074/ 500347.20059

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that I verily believe that I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

LASER-COMPATIBLE NIR-MARKER DYES

the specification of which

☐ is attached hereto.

☐ was filed on __________ as United States patent application Serial Number __________

☒ was filed on March 9, 2000 as PCT international patent application No. PCT/DE 00/00802
and was amended on __________ (if any).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information known to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. 119
Germany	199 11 421.8	11 March 1999	YES X NO ___
			YES ___ NO ___

I hereby appoint REED SMITH LLP and the members of the firm: Lloyd McAulay, Reg. No. 20,423; J. Harold Nissen, Reg. No. 17,283; Jules E. Goldberg, Reg. No. 24,408; Gerald H. Kiel, Reg. No. 25,116; Eugene LeDonne, Reg. No. 35,930; Stephen Chin, Reg. No. 39,938; and Arthur Dresner, Reg. No. 24,403; as attorneys with full power of substitution and revocation to prosecute all business in the Patent & Trademark Office connected therewith and to receive all correspondence.

6.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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